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# *Hypochnicium pini*, a new corticioid basidiomycete in East Asia

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Abstract — A new species, *Hypochnicium pini,* is described based on morphological and molecular characters. This species was collected in Seoul, Korea, and Hubei, China. It belongs to the *H. punctulatum* group and has a morphology quite similar to its sister taxon, *H. cremicolor*. However, the basidiome thickness, cystidial size, and ITS region sequences differentiate *H. pini* from *H. cremicolor*. Detailed description and illustration of *H. pini* are presented and a key to Korean species of *Hypochnicium* is provided.

Key words — phylogeny, *Polyporales*, taxonomy

## Introduction

*Hypochnicium* J. Erikss. is a basidiomycete genus of wood-rotting fungi. It is characterized by resupinate effused adnate basidiomes, a margin that is usually not especially differentiated, monomitic hyphal system, hyaline thin- to thick-walled hyphae, cystidia present or absent, more or less suburniform 4-spored basidia normally with a basal clamp, and ellipsoid to subglobose smooth or ornamented basidiospores with thickened cyanophilous walls (Eriksson & Ryvarden 1976). More than 30 species have been described worldwide, and a core group around the type, *H. bombycinum* (Sommerf.) J. Erikss., has been shown to be monophyletic when *Hyphoderma* species are used as outgroup (Paulus et al. 2007; Tellería et al. 2010).

Traditionally, northern European *Hypochnicium* species were subdivided into six morphological groups (Eriksson & Ryvarden 1976), and within each group, species delimitation was troublesome because of rather similar morphological features. Recently, molecular phylogenetic analysis has been used to delimit species in *Hypochnicium*. Nilsson & Hallenberg (2003) first used ITS region sequences to differentiate species within the *H. punctulatum* group [*H. albostramineum* (Bres.) Hallenb., *H. cremicolor* (Bres.) H. Nilsson & Hallenb., *H. punctulatum* (Cooke) J. Eriksss., *H. wakefieldiae* (Bres.) J. Eriksss.] and clearly distinguished the species which complemented morphology and crossing test results. Several additional species belonging to this complex have been described: *H. cystidiatum* Boidin & Gilles from Africa (Boidin & Lanquetin 1971), *H. aotearoae* B.C. Paulus et al. from New Zealand (Paulus et al. 2007), *H. guineense* Tellería et al. from Equatorial Guinea (Tellería et al. 2010), and *Hypochnicium patagonicum* Gorjón & Hallenb. from Chilean Patagonia (Gorjón & Hallenberg 2012).

Six *Hypochnicium* species have been reported from Korea: *H. bombycinum*, *H. detriticum* (Bourdot) J. Eriksss. & Ryvarden, *H. eichleri* (Bres. ex Sacc. & P. Syd.) J. Eriksss. & Ryvarden, *H. lundellii* (Bourdot) J. Eriksss., *H. punctulatum*, and *H. vellereum* (Ellis & Cragin) Parmasto (Jung 1991, 1994, 1996; Lim et al. 2010). *Hypochnicium eichleri* and *H. punctulatum* were reported by Jung (1991, 1996), who identified the species referring to *Corticiaceae* of North Europe (Eriksson & Ryvarden 1976). However, Nilsson & Hallenberg (2003) proved that "*H. punctulatum*" as described in Eriksson & Ryvarden (1976) was actually *H. cremicolor* (The type of *H. punctulatum* has bigger basidiospores than *H. cremicolor*). Moreover, *H. eichleri* has been synonymised with authentic *H. punctulatum*. Therefore the Korean report of "*H. punctulatum*" should be corrected to *H. cremicolor*; and the Korean record of *H. eichleri* should be updated to *H. punctulatum.*

Recently, the national biological inventory project (governed by the National Institute of Biological Resources) is underway in Korea. During the investigation of indigenous fungi, we found a *Hypochnicium* specimen sharing features with *H. cremicolor* but with smaller cystidia. Our further investigation revealed two specimens from China with the same deviating characteristics. Based on morphological and molecular data, we describe these three specimens as a new species.

## Materials & methods

### Collection and morphological examination

The corticioid basidiome was collected from Gungsan (Mt.), Seoul, Korea and air-dried. The dried specimen was used for macro- and microscopic examination. The specimen was mounted in 1% cotton blue, 5% KOH, 1% phloxine, and Melzer's

reagent (IKI) (Largent et al. 1977) and observed at up to 1000× using Olympus BX51 light microscope. Munsell color (2009) was followed as the color standard. More than 30 basidiospores were examined. The specimen was deposited at National Institute of Biological Resources, Korea (KB). The Chinese specimens were borrowed from National Museum of Natural Science, Taiwan (TNM) and examined as above.

### Phylogenetic analysis

For the Korean specimen, genomic DNA extraction, ITS region PCR, and sequencing were carried out according to Jang et al. (2012). For the Chinese specimens, the revised method of Cenis (1992) was used for genomic DNA extraction. PCR and sequencing were performed according to Jang et al. (2012). The sequences obtained in this study were deposited under the GenBank accession no. JX217823, KC282470–282472. The sequences were proofread and compared with the reference sequences in GenBank. Closely related *Hypochnicium* sequences were retrieved from GenBank. Only one sequence was selected when two or more sequences had the same nucleotides; *H. geogenium* (Bres.) J. Erikss. (GenBank AF429426) was included as outgroup based on the phylogenetic tree in Tellería et al. (2010). The sequences were aligned using MAFFT 6.885 (Katoh & Toh 2008) with L-INS-i method. The aligned dataset was viewed and manually edited using MacClade 4.08 (Maddison & Maddison 2005). Phylogenetic tree was constructed using MrBayes 3.2 (Ronquist et al. 2012). Two independent runs with 8 chains were operated with 1,000,000 generations and every 100th tree was saved. In this process, the best-fit model was searched by MrModeltest 2.3 (Nylander 2004) under AIC criterion and GTR + I model was applied. The graphical presentation of log likelihood value was checked and the first 25% of the trees were discarded. The 50% majority rule consensus tree was produced with the rest 75% of the trees. The potential scale reduction factors (PSRF) were reasonably close to 1.0 for all parameters. Posterior probabilities were calculated to support nodes. The tree was viewed using FigTree 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/).

## Taxonomy

***Hypochnicium pini*** Y. Jang & J,J. Kim, **sp. nov.** Figs 1–3

MycoBank MB 800807

Differs from *Hypochnicium cremicolor* by its thinner basidiome and its smaller cystidia.

Type: Korea. Seoul, Gangseo-gu, Gungsan (Mt.), 37°34′25″N 127°50′25″E, on bark of *Pinus densiflora* Siebold & Zucc., 23 Sep 2008, Yeongseon Jang KUC20081023-04 (**Holotype**, KB NIBRFG0000107453; GenBank JX217823).

Etymology: Growing on pines (*Pinus*).

Basidiome resupinate, effused, membranaceous, pale yellow (2.5Y8/2, 2.5Y8.5/2) when dry, up to 0.1 mm thick; hymenophore smooth to porulose (hypochnoid); margin not differentiated. Hyphal system monomitic; hyphae with clamps, ramified, hyaline, 3.8–5.8 µm wide; subhymenial hyphae thin-walled while the basal ones are thick-walled, walls up to 1.5 µm thick, loosely interwoven. Cystidia subcylindrical, slightly tapering but with an obtuse apex, enclosed

Fig. 1. *Hypochnicium pini* (NIBRFG0000107453, holotype).
Basidiocarp. Scale bar = 1 cm.

within the hymenium or projecting up to 40 µm above the basidial layer, thin-walled, variable in size, 40–80 × 5.5–8.5 µm. Basidia clavate, sinuous, 28–39 × 5.5–7.5 µm, with four sterigmata and a basal clamp. Basidiospores broadly ellipsoid to subglobose, 5.5–6.9 × 4.6–5.9 µm, mean 6.1 × 5.2 µm, verrucose in 1% cotton blue and 1% phloxine, smooth in 5% KOH, thick-walled, some with a few guttula, spore wall cyanophilous, IKI–.

Ecology & distribution — China and Korea, on *Pinus* spp.

Additional specimens examined:

***Hypochnicium pini***: **China**, **Hubei**, Wuhan, Mt. Lion, alt. 30 m, 30°29′N 114°21′E, on trunk of *Pinus* sp., 12 November 2008, S.H. Wu 0811-75 (TNM F0023763; GenBank KC282471); Wu 0811-77 (TNM F0023765; GenBank KC282472).

***Hypochnicium cremicolor***: **China**, **Yunnan**, Tali, Tsangshan, alt. 2800 m, on trunk of *Pinus armandii* Franch., 19 October 2000, S.H. Wu & S.C. Wu 0010-180 (TNM F0012210; GenBank KC282470).

Remarks — *Hypochnicium pini* is very similar to *H. cremicolor*. However, *H. cremicolor* has a thicker basidiome (0.1–0.3 µm) and larger cystidia (70–150 × 7–10 µm; Eriksson & Ryvarden 1976, as "*H. punctulatum*"). Phylogenetic analysis also revealed the close relationship between the two species, but there was sufficient genetic differentiation to separate them (Fig. 3).

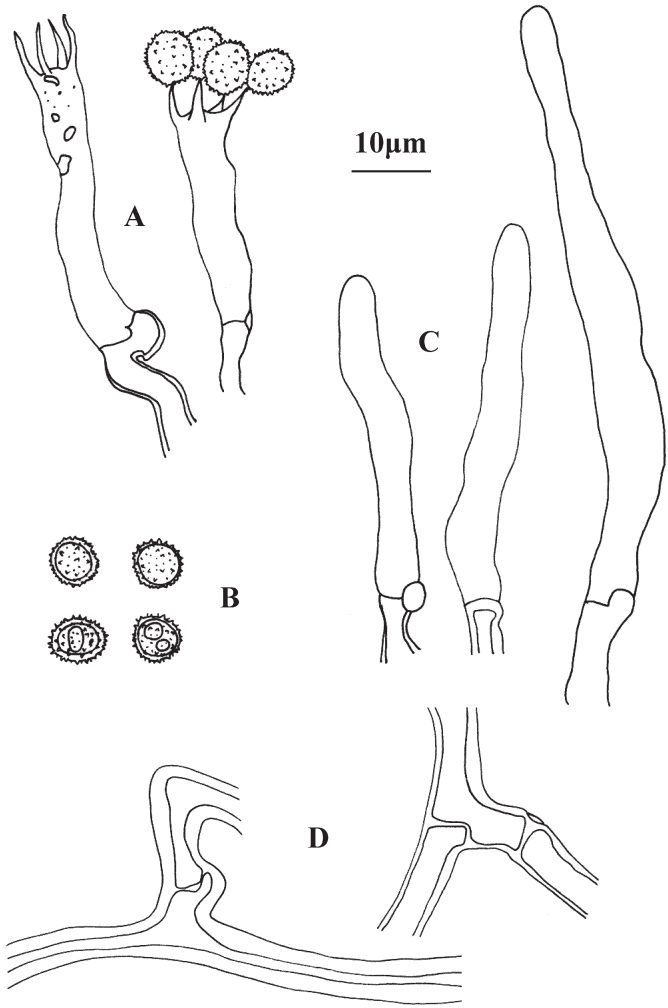


Fig. 2. *Hypochnicium pini* (NIBRFG0000107453, holotype).
A, basidia; B, basidiospores; C, cystidia; D, thick-walled basal hyphae.

**Phylogeny**

The amplified ITS region sequences of *Hypochnicium pini* NIBRFG0000107453 from Korea, and F0023763 and F0023765 from China were 578 bp. There were two nucleotide differences between the Korean specimen and the Chinese specimens of *H. pini* and the sequences of Chinese specimens were identical.

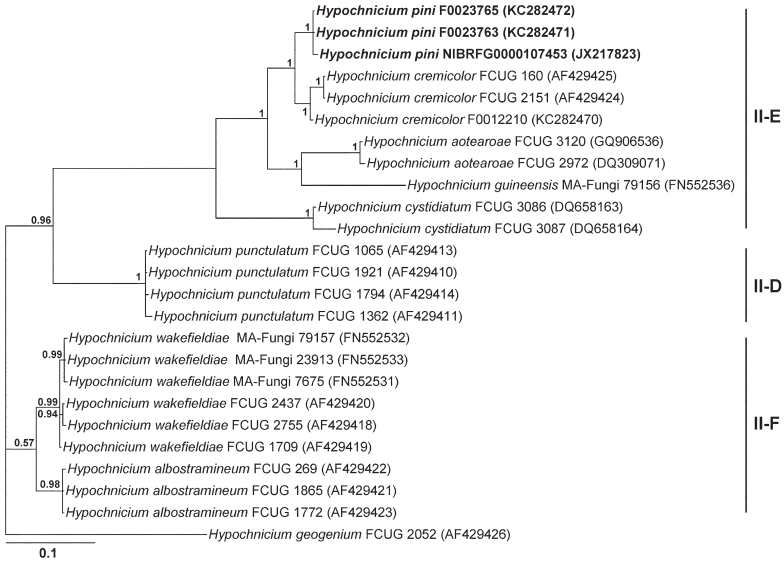
Hypochnicium pini F0023765 (KC282472)
Hypochnicium pini F0023763 (KC282471)
Hypochnicium pini NIBRFG0000107453 (JX217823)
Hypochnicium cremicolor FCUG 160 (AF429425)
Hypochnicium cremicolor FCUG 2151 (AF429424)
Hypochnicium cremicolor F0012210 (KC282470)
Hypochnicium aotearoae FCUG 3120 (GQ906536)
Hypochnicium aotearoae FCUG 2972 (DQ309071)
Hypochnicium guineensis MA-Fungi 79156 (FN552536)
Hypochnicium cystidiatum FCUG 3086 (DQ658163)
Hypochnicium cystidiatum FCUG 3087 (DQ658164)
Hypochnicium punctulatum FCUG 1065 (AF429413)
Hypochnicium punctulatum FCUG 1921 (AF429410)
Hypochnicium punctulatum FCUG 1794 (AF429414)
Hypochnicium punctulatum FCUG 1362 (AF429411)
Hypochnicium wakefieldiae MA-Fungi 79157 (FN552532)
Hypochnicium wakefieldiae MA-Fungi 23913 (FN552533)
Hypochnicium wakefieldiae MA-Fungi 7675 (FN552531)
Hypochnicium wakefieldiae FCUG 2437 (AF429420)
Hypochnicium wakefieldiae FCUG 2755 (AF429418)
Hypochnicium wakefieldiae FCUG 1709 (AF429419)
Hypochnicium albostramineum FCUG 269 (AF429422)
Hypochnicium albostramineum FCUG 1865 (AF429421)
Hypochnicium albostramineum FCUG 1772 (AF429423)
Hypochnicium geogenium FCUG 2052 (AF429426)
II-E
II-D
II-F
0.1

Fig. 3. 50% majority rule consensus tree of the *Hypochnicium punctulatum* group inferred by ITS region sequences using bayesian analysis. The tree was rooted by *H. geogenium* FCUG 2052. Numbers above branches indicate posterior probabilities. *H. pini* is indicated by bold type. The clades presented in the tree follow those resolved by Telleria et al. (2010).

By the nucleotide BLAST, the closest match was *H. cremicolor* FCUG160 from Denmark AF429425 (93%) and *H. cremicolor* FCUG2151 from Spain AF429424 (93%) (555 out of 596 positions are identical for Korean specimens and 557 out of 596 positions for Chinese specimens). In order to fully understand the relationship within the *H. punctulatum* group, *H. pini* sequences were aligned with 22 closely related *Hypochnicium* sequences obtained from GenBank including *H. cremicolor* Wu 0010-180 from China, and with *H. geogenium* FCUG2052 as outgroup. The aligned ITS dataset of 25 taxa comprised 586 characters including gaps. The 50% majority rule consensus tree using bayesian analysis is shown in Fig. 3.

Each species was well resolved in the tree as indicated by high posterior probability values. Three main groups were recovered which correspond to the subclades II-D, II-E, and II-F in Tellería et al. (2010). They all have ornamented basidiospores, but there are just minute morphological characteristics which distinguish each subclade. *Hypochnicium pini* was included in subclade II-E with four formerly described species – *H. aotearoae*, *H. cremicolor*, *H. guineense*, and *H. cystidiatum*. *Hypochnicium pini* NIBRFG0000107453 from

Korea was monophyletic with *H. pini* F0023763 and F0023765 from China with high posterior probability (1.0 p.p.); and *H. cremicolor* Wu 0010-180 from China was monophyletic with formerly sequenced *H. cremicolor* FCUG160 and FCUG 2151, also with high support (1.0 p.p.), and it was 98% similar to them in BLAST search (588 out of 596 positions are identical).

## Discussion

Morphologically, species within II-E clade have similar characteristics. *Hypochnicium pini* is characterized by its pale yellow basidiocarp, hypochnoid hymenophore, thick-walled basal hyphae, one type of cystidium and broadly ellipsoid to subglobose spores, which are very similar to *H. cremicolor* (Figs 1, 2). They both have the basal thick-walled hyphae, one type of cystidium, and ornamented basidiospores which are similar in size [*H. pini*: 5.5–6.9 × 4.6–5.9 µm; and *H. cremicolor*: (5–)5.5–6.5 (–7.5) × 4.5–5 µm (Eriksson & Ryvarden 1976) or 6–6.5 × 5–5.5 µm (Nilsson & Hallenberg 2003)]. The hosts of *H. pini* are *Pinus* spp. while *H. cremicolor* has been reported from both broadleaved and coniferous trees including *Pinus* spp. (Eriksson & Ryvarden 1976, Bernicchia & Gorjón 2010). The obvious morphological differences are the thickness of basidiome and the size of cystidia (Table 1). *Hypochnicium aotearoae* and *H. cystidiatum* differ from *H. pini* by having two types of cystidia, and *H. guineense* differs by larger basidiospores (Tellería et al. 2010).

Table 1. Morphological characteristics of Korean species of *Hypochnicium* s.l.

| Species | Cystidia (µm) | Basidia (µm) | Basidiospores (µm) |
|---|---|---|---|
| *H. bombycinum* (Jung 1994) | None | 40–50 × 6–8 | Smooth, 8–12 × 6–8 |
| *H. cremicolor* (Jung 1996, as "*H. punctulatum*") | 60–130 × 9–12 | 25–30 × 6.5–7.5 | Ornamented, (5–)6–6.5 × 4.5–5.5 |
| *H. detriticum* (Jung 1994) | 60–100 × 6–10 | 15–20 × 5 | Smooth, 4.5–6 × 3.5–4 |
| *H. lundellii* (Jung 1994) | None | 25–30 × 5–6 | Smooth, 5.5–6 × 4.5–5 |
| *H. pini* (this paper) | 40–80 × 5.5–8.5 | 28–39 × 5.5–7.5 | Ornamented, 5.6–6.9 × 4.8–5.9 |
| *H. punctulatum* (Jung 1991, as "*H. eichleri*") | 90–110 × 6–10 | 25–35 × 7.5–9 | Ornamented, 8–9(–10) × 6–7.5 |
| *H. vellereum* (Lim et al. 2010) | None | 42–65 × 4–7.42 | Ornamented, 6.8–7.4 × 5.7–6.2 |

In conclusion, we propose that the unknown *Hypochnicium* species collected from Gungsan (Mt.) in Korea and from Hubei in China represents a new species, *Hypochnicium pini*, minutely distinguished by morphological data, but with distinct genetic differences towards other species in this complex. We also update the list of Korean species of *Hypochnicium* sensu lato (Table 1).

## Key to the Korean species of *Hypochnicium* s.l.

1. Without cystidia . . . . . 2
1. With cystidia . . . . . 4
2. Spores smooth . . . . . 3
2. Spores globose, with irregularly uneven or finely rough surface . . . . . *H. vellereum*
3. Spores broadly ellipsoid to ovoid, 8–12 × 6–8 µm . . . . . *H. bombycinum*
3. Spores ellipsoid, 5.5–6 × 4.5–5 µm . . . . . *H. lundellii*
4. Spores smooth . . . . . *H. detriticum*
4. Spores ornamented . . . . . 5
5. Spores longer than 8 µm. . . . . *H. punctulatum*
5. Spores shorter than 8 µm . . . . . 6
6. Basidiome 0.1–0.3 mm thick, cystidia 60–130 × 9–12 µm . . . . . *H. cremicolor*
6. Basidiome <0.1 mm thick, cystidia 40–80 × 5.5–8.5 µm. . . . . *H. pini*

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